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Abstracts

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Uncovering of Classical Swine Fever Virus adaptive response to vaccination by Next Generation Sequencing

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Next Generation Sequencing (NGS) has rapidly become the preferred technology in nucleotide sequencing, and can be applied to unravel molecular adaptation of RNA viruses such as Classical Swine Fever Virus (CSFV). However, the detection of low frequency variants within viral populations by NGS is affected by errors introduced during sample preparation and sequencing, and so far no definitive solution to this problem has been presented. Here we present NGS data from an immunisation/challenge experiment obtained by two different NGS-platforms (Ion PGM and Roche FLX). The pigs were immunised with the DIVA vaccine candidate, vR26_E2gif, and subsequently challenged with the highly virulent CSFV strain “Koslov”. NGS data of Koslov RNA derived from serum of vaccinated pigs and mock-vaccinated controls was obtained and analysed. Before low frequency analysis, we performed a rigorous error correction. Several tools were benchmarked and the RC454 developed by the Broad institute outperformed the others by removing most indels from detection and strengthened the variation detection limit. Subsequently, variation analysis revealed significant differences between the CSFV sequence data from the vaccinated and the mock-vaccinated groups. The viral sequences obtained from the mock-vaccinated pigs had a similar single-nucleotide polymorphism (SNP) distribution as the challenge virus, which was not the case for the sequence data from the vaccinated group where a complete change of the SNP distribution was observed. Additionally, new detectable non-synonymous SNPs were found in the vaccinated pigs indicating selection pressure onto the challenge virus, which was not observed in the mock-vaccinated group.